https://doi.org/10.22319/rmcp.v12i4.5739

Article

Frequency of serum antibodies against infectious bovine rhinotracheitis and bovine viral diarrhea viruses in bulls, and their relationship with the presence of the viruses in semen

Jorge Víctor Rosete Fernández [a]

Guadalupe A. Socci Escatell [b]

Abraham Fragoso Islas [a]

Juan Prisciliano Zárate Martínez [c]

Sara Olazarán Jenkins [a]

Lorenzo Granados Zurita [d]

Ángel Ríos Utrera [c]*

[a] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Sitio Experimental Las Margaritas. Kilómetro 9.5 carretera Hueytamalco-Tenampulco, Hueytamalco, Puebla, México.

[b] INIFAP, CENID Salud Animal e Inocuidad. Ciudad de México, México.

[c] INIFAP, Campo Experimental La Posta. Veracruz, México.

[d] INIFAP, Campo Experimental Huimanguillo. Tabasco, México.

*Corresponding author: rios.angel@inifap.gob.mx

Abstract:

The objective was to estimate the frequency of serum antibodies against infectious bovine rhinotracheitis (IBRV) and bovine viral diarrhea (BVDV) viruses in unvaccinated bulls, as well as the relationship between the presence of antibodies in serum and the presence of these viruses in semen. Antibodies were detected by ELISA, while the presence of the viruses in semen by PCR. Logistic regression analyses were performed with the PROC GENMOD of SAS. The factors were: state, herd nested in state, and genotype of the bull (except for the presence of the viruses in semen). The degree of association between the presence of serum antibodies and the presence of the viruses in semen was measured by the phi (r) correlation. None of the three factors were significant (P>0.05). For IBRV, the frequency of serum antibodies by state ranged from 66 to 86 %, while by herd, it ranged from 28 to 90 %. For BVDV, the frequency of serum antibodies by state ranged from 58 to 76 %, while by herd, it ranged from 43 to 86 %. The presence of IBRV in semen, by state, ranged from 50 to 55 %, while by herd, it ranged from 33 to 80 %. No association (P>0.05) was found between the presence of antibodies in serum and the presence of IBRV (r=0.07) and BVDV in semen (r=0.16). The presence of serum antibodies suggests infection of bulls, but the presence of the viruses in semen suggests their transmission by sexual contact.

Key words: Bulls, Infectious bovine rhinotracheitis, Bovine viral diarrhea, Antibodies, Antigens, Semen, Tropics.

Received: 20/07/2020

Accepted: 30/03/2021

Introduction

Bovine viral diarrhea (BVD) is an acute and epizootic disease[1] because it causes a wide range of lesions and clinical manifestations, as well as

considerable losses in beef and dairy cattle[2], with reproductive disorders being the ones with the greatest economic impact[3]. Due to its pathogenesis, BVD has been considered the most complicated viral disease in cattle[4].

There are two biotypes of the BVD virus (BVDV): the cytopathic (CP) and the non-cytopathic (NCP), according to their behavior in cell cultures[2,4,5]. The common biotype in most (95 %) field isolates is NCP; the CP biotype is generated by mutations or rearrangements of the genome of the original paternal NCP strain. In addition, due to their genetic-antigenic characteristics, they are classified into two genotypes, which are BVDV-1 and BVDV-2, which are mostly NCP. These biotypes and genotypes are independent qualities of Pestiviruses[6].

Each biotype has a specific role in a variety of clinical syndromes, such as chronic, acute, and congenital infections. BVDV-2 has been associated with outbreaks of severe acute infections and hemorrhagic syndrome[7]. The main characteristic of this virus is its genetic and antigenic variability, since RNA viruses are characterized by their plasticity, which is due to the lack of an efficient exonuclease to correct poorly incorporated bases, causing a high-frequency base substitution (one error per 10,000 polymerized nucleotides). BVDV uses this strategy to survive, causing mutant strains that escape the host's immune response.

BVDV mainly infects cattle, a species for which it represents one of the most important pathogens, but it can also be found in sheep, goats[1], pigs, alpacas, llamas, camels, water buffaloes and wild ruminants[3]. This peculiarity must be taken into account when implementing a control program, since Pestiviruses cross the species barrier[3]. The great diversity of studies available indicates that BVD has a worldwide distribution[6,8-11], since the virus is of high morbidity and low mortality[12], in such a way that a persistently infected animal less than four months old is able to infect 90 % of its herd mates in housed conditions[13].

For its part, infectious bovine rhinotracheitis (IBR) is a disease distributed in various regions of Mexico, since neutralizing antibodies against the IBR virus (IBRV) have been found since 1988 in cattle from Estado de México, Puebla

and Yucatán, from cattle with respiratory signs that suggested the presence of the virus[14]. In Tizimín, Yucatán, a seroprevalence of 5.33 %[15] was observed and, subsequently, in another study in cattle without a history of vaccination, a seroprevalence of 54.4 %[16] was found, which shows that IBRV is present and latent in the tropics, since its prevalence has been increasing, because it is highly contagious.

IBR manifests itself in different ways and is transmitted by direct contact with nasal, ocular and genital secretions, as well as with fresh semen from infected bulls[17]. This disease has been little studied in bulls, mainly in commercial herds from the Mexican tropics, because, although reproductive failures have been reported in cows, no action has been taken to identify health problems in bulls. Therefore, it is important to study it, since in most cows, the disease goes unnoticed because it does not cause death, having abortion as its main characteristic, affecting reproductive and productive parameters and significantly increasing economic losses[18], since there is less production of fattening calves and replacement heifers.

Some studies have shown the presence of IBRV in frozen semen. In artificial insemination centers, isolates of this agent have been carried out in clinically healthy bulls[19], but little research has been done on the presence of this virus in the semen of bulls that reproduce by means of natural mounting in commercial herds from the Mexican tropics. In previous studies, it is recognized that infected bulls are a risk factor for the transmission of IBR, since its causative agent is transmitted by venereal route[20], reactivating the disease in the herd and keeping it infected, which explains the high antibody titers found in some studies in bulls destined for natural mounting[21]. In herds where reproduction has been through natural mounting, prevalences of 74.0[22] and 69.5 %[23] have been found, revealing the participation of the male in the transmission of the disease, despite the fact that serological tests could be negative[21]. In one study, it was observed that the fertility of bulls was not affected by IBR[24]; therefore, it is a risk factor that should be considered when acquiring bulls for mounting without sanitary control, which undoubtedly contributes to worsening the situation of IBR in herds.

Based on the above, the objective of the present work was to estimate the frequency of serum antibodies against BVD and IBR viruses in unvaccinated bulls, as well as the relationship between the presence of antibodies in serum and the presence of the viruses in semen.

Material and methods

The study was conducted in 14 commercial herds located in the tropics and subtropics of Mexico in the states of Puebla, Tabasco and Veracruz. The herds are located in the area of influence of INIFAP's experimental stations: Las Margaritas, in Puebla, Huimanguillo, in Tabasco, and La Posta, in Veracruz.

The herds were selected based on a non-probabilistic convenience sampling, according to the farmers' interest in participating in the present study. On the other hand, the sample size (n= 76) depended on the existing bulls in each participating herd. The bulls belonged to herds officially free of *Brucella abortus* and *Mycobacterium bovis*, which were destined for beef production and dual purpose. The genotype of the bulls was classified into zebu (*Bos indicus*), European (*Bos taurus*) and crossed (*Bos taurus* x *Bos indicus*).

The blood samples were obtained from the coccygeal vein and kept between 4 and 6 °C in a cooler until reaching the laboratory of the corresponding experimental station, where they were centrifuged at 4,000 rpm for 10 min to obtain 3 ml of serum/bull. Serum samples were stored in polyethylene vials at -20 °C until the time of analysis. The serological diagnosis for the detection of antibodies against IBR and BVD viruses was made with the CIVTEST BOVIS IBR and CIVTEST BOVIS BVD/BD P80 kits (Laboratorios Hipra, S.A., Mexico), based on the ELISA test, whose sensitivity and specificity is 96.3 and 99.5 %, respectively. In both serological diagnostics, the reading was made at an optical density of 450 nanometers in an ELx800 spectrophotometer, BioTek brand (BioTek Instruments, Inc., USA).

The semen samples (3 ml/bull) were obtained by means of electroejaculation and contained in polyethylene tubes; subsequently, they were kept between 4

and 6 °C in a cooler. Upon arrival at the laboratory, the samples were kept in freezing at -20 °C until the time of analysis. These samples were analyzed by PCR to detect antigens of the viruses mentioned. From each semen sample already homogenized, 200 µl were taken for the extraction of nucleic acids by means of the commercial High Pure PCR Template Preparation Kit (Laboratorios Roche), under the protocol described by the manufacturer for whole blood samples, with the modification that, in the last step, the nucleic acids were eluted in 50 µl of bidistilled injectable water. DNA amplification for IBR virus detection was performed using a pair of primers that amplify 468 bp of the glycoprotein gI gene[25]. The reaction mixture was carried out in a final volume of 25 µl with the following concentrations: 1X of 10X Buffer, 1.5 mM of $MgCl_2$, 0.4 mM of dNTPs, 20 pmol of each primer, 1.25 U of Taq polymerase and 1.5 mcg of BSA. The amplification program consisted of 1 cycle at 95 °C for 1 min; 35 cycles at 95 °C for 1 min, 62 °C 1 min and 72 °C 1 min; and a final cycle at 72 °C for 7 min. Electrophoresis of the amplification products was carried out in 1.5 % agarose gels, stained with the GelRed reagent (Biotium). The visualization of the amplification products was done under UV light on a photodocumenter.

For the detection of the BVD virus, the synthesis of cDNA and its amplification were carried out in a single step. A pair of primers that amplify a 191 bp fragment of the 5' UTR region of the viral genome were used[26]. The reaction was carried out in a final volume of 25 µl under the following conditions: 1X of 10X Buffer, 1.5 mM of $MgCl_2$, 0.4 mM of dNTPs, 20 pmol of each primer, 1.25 U of Taq polymerase, 6 U of reverse Transcriptase and 1.5 mcg of BSA. The amplification program consisted of 1 cycle at 48 °C for 30 min, followed by 1 cycle at 95 °C for 10 min; 35 cycles at 94 °C for 1 min, 58 °C for 1 min, 72 °C for 1 min; and a final extension cycle of 72 °C for 7 min. Electrophoresis and visualization were performed in the same way as for the IBR virus.

The frequencies of antibodies in serum, as well as the frequencies of antigens in semen, were treated as binary characteristics, so they were recorded as 1 when a bull tested positive for ELISA or PCR test, respectively; otherwise, as 0. The statistical model (binomial logistic regression model) to analyze antibody frequencies included the factors state of the Mexican Republic, herd

nested in the state and genotype of the bull; to analyze the frequencies of antigens in semen, the statistical model only included state of the Mexican Republic and herd nested in the state of the Mexican Republic. Logistic regression analyses by characteristic were performed with the GENMOD procedure of the SAS package, using a logit link function for the binomial distribution. The convergence criterion applied in each statistical analysis was 10^{-8}.

The degree of association between the presence of antibodies in serum and antigens in semen of BVD and IBR viruses, as an indicator of the elimination of viruses through semen, was determined with the phi coefficient, also called the Mathews correlation coefficient, which is calculated for 2x2 contingency tables, positive (1) or negative (0) bulls for the presence of antibodies in serum, and positive (1) or negative (0) bulls for the presence of antigens in semen. The correlation coefficients, as well as the statistical significance for determining whether they were different from zero, were estimated with the CORR procedure of the SAS package.

Results and discussion

None of the three adjustment factors affected ($P>0.05$) the frequency of serum antibodies against BVDV. The frequency of BVDV antigens in semen was not estimable, since a large proportion of the bulls were negative to the PCR test (97.4 %), resulting in the absence of variation in some of the adjustment factors included in the statistical model. The frequencies of serum antibodies against BVDV and their 95 % confidence intervals, by state and genotype, are shown in Tables 1 and 2, respectively; however, frequencies by herd are not presented. The frequency of serum antibodies against BVDV ranged from 58 to 76 % by state, from 43 to 86 % by herd, and from 54 to 79 % by genotype. No association ($P>0.05$) was found between the presence of antibodies in serum and the presence of BVDV in semen (r= 0.16).

Table 1: Frequencies (± standard errors) of serum antibodies against bovine viral diarrhea virus and 95 % confidence intervals, by state

State	No. of bulls	Frequency, %	Interval
Puebla	28	58 ± 11	36 - 77
Tabasco	26	59 ± 11	38 - 78
Veracruz	22	76 ± 11	49 - 92

(P>0.05).

Table 2: Frequencies (± standard errors) of serum antibodies against bovine viral diarrhea virus and 95 % confidence intervals, by genotype

Genotype	No. of bulls	Frequency, %	Interval
Zebu	29	54 ± 13	29 - 77
Cross	33	60 ± 10	40 - 77
European	14	79 ± 14	43 - 95

(P>0.05).

The frequency of serum antibodies against BVDV estimated in the present study is relatively high and, consequently, of consideration. Therefore, knowing in advance that, in the herds evaluated, the reproduction was carried out by natural mounting, the bulls represent a risk factor in the transmission of BVD. On the other hand, although the frequency of BVDV antigens in semen could not be estimated using a statistical model, it is considered low, since a large proportion of bulls were negative to the PCR test (97.4 %), which suggests that not all bulls were eliminating the virus at the time of taking the semen sample, or, there is a possibility that only persistently infected (PI) bulls eliminated the virus, as the virus has been shown to replicate in the prostate and seminal vesicles in PI bulls and is not constantly eliminated[27,28,29]. In another study in bulls from Peru, a relatively high frequency of serum antibodies was also observed (51.3 %), arguing a wide diffusion and viral activity among animals and emphasizing the risk of transmission through semen[29], so vaccination in females is important to prevent reproductive problems due to the risk of infection[27].

The frequency of serum antibodies against BVDV found in this study suggests that the herds to which the bulls belong are infected and permanently exposed to reinfection, and as for the frequency of BVDV in semen, it was expected

that this would be much higher, and that it would show a high correlation with the presence of antibodies in serum, but this did not happen probably because BVDV is not constantly eliminated through semen[29]; however, even the minimal presence of BVDV in semen represents a risk factor at the time of natural mounting, as other authors have argued[28,30], and if it is frozen semen, it must be harmless, because infecting inseminated cows causes fertility problems[31-33], due to the risk of elimination of the virus, although not permanently in that ejaculated by PI bulls[28,29,34]. This is demonstrated by a study in two-year-old bulls, with no history of BVD and inoculated nasally with BVDV[35], which eliminated the virus in semen for a period of up to seven months, and then it disappeared, which may explain why very few bulls eliminated the virus in semen in the present study, since it is not constantly eliminated[29,34,35]. Even so, the detection of BVDV in serum or semen is important in sires for natural mounting or semen freezing, because by preventing the use of positive animals, the risk of transmission to females is minimized[28,30].

Because BVD persists in infected animals, its transmission to healthy animals is facilitated[3,7,36,37], so vaccination is the appropriate control tool, as the elimination of PI animals is complicated and requires a lot of time. Therefore, the herds evaluated in this study should be vaccinated[27] to prevent reproductive problems[29].

For the frequency of serum antibodies against IBRV, state, herd nested in state and genotype of the bull were also not important ($P>0.05$) adjustment factors. Tables 3 and 4 show the frequencies of serum antibodies against IBRV and their 95 % confidence intervals, by state and genotype of the bull, respectively; however, the frequencies by herd are not shown. The frequency of serum antibodies against this virus ranged from 66 to 83 % by state, from 28 to 90 % by herd, and from 54 to 84 % by genotype.

Table 3: Frequencies (± standard errors) of serum antibodies against infectious bovine rhinotracheitis virus and 95 % confidence intervals, by state

State	No. of bulls	Frequency, %	Interval
Puebla	34	83 ± 7	63 - 93
Tabasco	11	66 ± 18	29 - 90
Veracruz	29	70 ± 10	47 - 86

(P>0.05).

Table 4: Frequencies (± standard errors) of serum antibodies against infectious bovine rhinotracheitis virus and 95 % confidence intervals, by genotype

Genotype	No. of bulls	Frequency, %	Interval
Zebu	33	79 ± 10	52 - 92
Cross	29	54 ± 13	30 - 76
European	12	84 ± 12	46 - 97

(P>0.05).

The presence of IBRV in semen was also not affected by the effects of state and herd; the corresponding frequency ranged from 50 to 55 % by state (Table 5) and from 33 to 80 % by herd (these frequencies are not presented). The presence of antibodies in serum and the presence of IBRV in semen were also not correlated (r=0.07; P>0.05).

Table 5: Frequencies (± standard errors) for infectious bovine rhinotracheitis virus in semen and 95 % confidence intervals, by state

State	No. of bulls	Frequency, %	Interval
Puebla	21	54.9 ± 12.5	31 - 77
Tabasco	12	50.0 ± 15.3	23 - 77
Veracruz	23	52.3 ± 10.4	33 - 71

(P>0.05).

The results of this study show a relatively high frequency of serum antibodies against IBRV. A similar (69.5 %) frequency was found in dual-purpose crossbred bulls from herds in the municipality of Tonalá, Chiapas[23], as well

as in dual-purpose crossbred bulls for beef production from herds in the Eastern Mountain range of Puebla (76.0 %)[24] and bulls for beef production in Yucatán (54.4 %)[16]. On the other hand, in bulls for beef production of herds from the Isthmus and the Coast of Oaxaca[38], lower frequencies were observed (31.6 and 27.9 %, respectively). In studies conducted in Colombian bulls, relatively high frequencies were observed, 85.5 % in Antioquia[20], 67.6 % in Urabá and 75.0 % in Valle del Cauca[39].

Regarding the presence of IBRV in semen, the identification of the virus has been documented in bulls for natural mounting, as well as in bulls of artificial insemination centers[40], in which both viruses have been isolated from frozen semen straws of several commercial houses[41], evidencing that artificial insemination is also an important risk factor in the spread of IBR, since it is a pathogen frequently present in semen, associated with low quality of it. The virus is found more in the seminal plasma than in the cell fraction, so that its transmission has shown loss of fertility in cows[31].

In this study, no association was found between the relatively high frequency of antibodies and the presence of IBRV in semen, in which there were only 53 % of bulls that eliminated the virus, in contrast to a previous study[40] in which of the total number of bulls seropositive to antibodies against the virus, 100 % eliminated it in semen. This may be due to factors that induce the excretion or reactivation of the virus, such as heat stress, handling stress, corticosteroid treatments, which cause immunosuppression, which allows the virus to replicate in epithelial cells and other tissues (ocular, for example), without reaching the reproductive organs; or, that the opposite happens, that the immune system is functioning perfectly, preventing the replication of the virus and, therefore, the virus is not in semen at the time of ejaculation[42-44]. However, the detection of antibodies against IBRV and antigens in semen, together or separately, are effective in detecting the disease in bulls[45] and, therefore, in the herd. Finally, due to the high frequencies of antibodies in serum and IBRV in semen in this study and considering that the disease is also transmitted by sexual contact, it is important to note that a control campaign for IBR in Mexico is urgent, as has been done in Brazil[46].

Conclusions and implications

No association was found between the presence of antibodies in serum and the presence of the viruses in semen; nor were differences found between states in the frequency of serum antibodies against IBR and BVD viruses or in the frequency of IBRV antigens in semen. However, these frequencies were of considerable magnitude, so a control program through vaccination should be implemented. If a bull eliminates these viruses through semen, it can be considered persistently infected, which should also be considered before buying bulls, which should be free of BVD and IBR, tested with laboratory tests.

Literature cited:

1. Obando RCA, Rodríguez JM. Diarrea viral bovina. En: González-Stagnaro C, Soto BE editores. Manual de ganadería doble propósito. 1ª ed. Maracaibo, Venezuela: Astro Data, S. A.; 2005:317-322.

2. Rondón I. Diarrea viral bovina: Patogénesis e inmunopatología. Rev MVZ Córdoba 2006;11(1):694-704.

3. Lértora WJ. Diarrea viral bovina: Actualización. Rev Vet FCV UNNE 2003;14(1):1-11.

4. Ramírez RR, Chavarría MB, López MA, Rodríguez TLE, Nevárez GAM. Presencia del virus de la diarrea viral bovina y su asociación con otros cuadros patológicos en ganado en corral de engorda. Vet Méx 2012;43(3):225-234.

5. Vargas DS, Jaime J, Vera JV. Perspectivas para el control del virus de la diarrea viral bovina (BVDV). Rev Colomb Cienc Pecu 2009;22:677-688.

6. Carter GR, Wise DJ, Flores EF. Flaviviridae. En: Carter GR, Wise DJ, Flores EF eds. Virología veterinaria. International Veterinary Information Service, Ithaca NY. 2005:251-262.

7. OIE. Office International des Epizooties. Diarrea Viral Bovina. Manual de la OIE Sobre Animales Terrestres. 2008:1-15.

8. Bedekovic T, Lemo N, Barbic L, Cvetnic Z, Lojkic I, Benic M, *et al*. Influence of category, herd size, grazing and management on epidemiology of bovine viral diarrhoea in dairy herds. Acta Vet Brno 2013;82:125-130.

9. Chowdhury MMR, Afrin F, Saha SS, Jhontu S, Asgar MA. Prevalence and haematological parameters for bovine viral diarrhoea (BVD) in South Bengal areas in Bangladesh. The Bangladesh Vet 2015;32(2):48-54.

10. Handel IG, Willoughby K, Land F, Koterwas B, Morgan KL, Tanya VN, *et al*. Seroepidemiology of bovine viral diarrhoea virus (BVDV) in the Adamawa region of Cameroon and use of the SPOT test to identify herds with PI calves. Plos One 2011;6(7):e21620.

11. Luzzago C, Lauzi S, Ebranati E, Giammarioli M, Moreno A, Cannella V, *et al*. Extended genetic diversity of bovine viral diarrhea virus and frequency of genotypes and subtypes in cattle in Italy between 1995 and 2013. BioMed Res Int 2014:1-8.

12. Gasque GR. Diarrea Viral Bovina. 1ª Ed. México, D.F.: UNAM; 2008.

13. Houe H. Survivorship of animals persistently infected with bovine viral diarrhoea virus (BVDV). Pre Vet Med 1993;15:275-283.

14. Correa, G. Complejo reproductivo bovino. En: Correa, G. Enfermedades virales de los animales domésticos poligástricos. 5ª ed. Paradigmas. México, D.F. 1988:45-90.

15. Calderón VG, Alvarado IA, Vilchis MC, Aguilar SA, Batalla CD. Detección de seropositividad al virus de rinotraqueitis infecciosa bovina (IBR) en ganado del municipio de Tizimín, Yucatán, México. Téc Pecu Méx 1997;35(3):161-164.

16. Solis-Calderon JJ, Segura VM, Segura JC, Alvarado IA. Seroprevalence of and risk factors for infection bovine rhinotracheitis in beef cattle herds of Yucatan, Mexico. Prev Vet Med 2003;57(4):199-208.

17. Nuotio L, Neuvonen E, Hyytiäinen M. Epidemiology and eradication of infectious bovine rhinotracheitis/infectious pustular vulvovaginitis (IBR/IPV) virus in Finland. Acta Vet Scand 2007;49(3):1-6.

18. Bracho CA, Jaramillo ACJ, Martínez, MJJ, Montaño HJA, Olguín BA. Comparación de tres pruebas diagnósticas para el aborto por rinotraqueitis infecciosa bovina en hatos lecheros. Vet Méx 2006;37:151-163.

19. Martínez CPJ, Riveira SIM. Antecedentes, generalidades y actualización en aspectos de patogénesis, diagnóstico y control de la diarrea viral bovina (DVB) y rinotraqueitis infecciosa bovina [tesis licenciatura]. Bogotá D.C., Colombia: Pontificia Universidad Javeriana; 2008.

20. Ruiz J, Jaime J, Vera VJ. Prevalencia serológica y aislamiento del Herpesvirus Bovino-1 (BHV-1) en hatos ganaderos de Antioquia y del Valle del Cauca. Rev Colomb Cienc Pecu 2010;23:299-307.

21. Schroeder WH. IBR-IPV y Reproducción. En: Schroeder WH editor. Fisiopatología reproductiva de la vaca. 1ª ed. Bogotá, Colombia: Celsus; 1999:825-833.

22. Romero-Salas D. Enfermedades que causan abortos en la ganadería bovina. 1ª ed. Veracruz, Veracruz: Universidad Veracruzana; 2012.

23. De los Santos MC, Orantes MA, Sánchez B, Manzur A, Cruz JL, Ruiz JL, *et al*. Determinación de anticuerpos de IBR mediante la técnica de ELISA en la zona Paredón- Boca del Cielo, Tonalá, Chiapas. Quehacer Cient Chis 2013;8(1):31-34.

24. Soto JR. Prevalencia de anticuerpos a IBR, BVD, Leptospirosis y Neosporosis y su relación con la fertilidad en sementales bovinos productores de carne del subtrópico húmedo de puebla [tesis licenciatura]. Puebla, México: Benemérita Universidad Autónoma de Puebla; 2014.

25. Deka D, Ramneek, Maiti NK, Oberoi MS. Detection of bovine herpesvirus-1 infection in breeding bull semen by virus isolation and polymerase chain reaction. Rev Sci Tech Off Int Epiz 2005;24(3):1085-1094.

26. Sandvik T, Paton DJ, Lowings PJ. Detection and identification of ruminant and porcine pestiviruses by nested amplification of 5' untranslated cDNA regions. J Virol Methods 1997;64:43-56.

27. González-Altamiranda EA, Kaiser GG, Weber N, Leunda MR, Pecora A, Malacan DA, *et al*. Clinical and reproductive consequences of using BVDV-contaminated semen in artificial insemination in a beef herd in Argentina. Anim Reprod Sci 2012;133(3-4):146-152.

28. Givens MD, Waldrop JG. Bovine viral diarrhea virus in embryo and semen production systems. Vet Clin Food Anim 2004;20(1):21-38.

29. Cárdenas AC, Rivera GH, Araínga RM, Ramírez VM, De Paz MJ. Prevalencia del virus de la diarrea viral bovina y de animales portadores del virus en bovinos en la provincia de Espinar, Cusco. Rev Inv Vet Perú 2011;22(3):261-267.

30. Mishra N, Kalaiyarasu S, Mallinath KC, Rajukumar K, Khetan RK, Gautam S, *et al*. Identification of bovine viral diarrhoea virus type 2 in cattle bull semen from southern India and its genetic characterization. Current Sci 2018;114(3):666-670.

31. Dejucq N, Jégou B. Viruses in the mammalian male genital tract and their effect on reproductive system. Microbiol Mol Biol R 2001;65(2):208-231.

32. Kirkland PD, McGowan MR, Mackintosh SG, Moyle A. Insemination of cattle with semen from a bull transiently infected with pestivirus. Vet Rec 1997;140:124-127.

33. Meyling A, Jensen AM. Transmission of bovine virus diarrhoea virus (BVDV) by artificial insemination (AI) with semen from a persistently-infected bull. Vet Microbiol 1998;17:97-105.

34. Niskanen R, Alenius S, Belák K, Baule C, Belák S, Voges H, Gustafsson H. Insemination of susceptible heifers with semen from a non-viraemic bull with persistent bovine virus diarrhoea virus infection localized in the testes. Reprod Dom Anim 2002;37:171-175.

35. Givens MD, Heath AM, Carson RL, Brock KV, Edens MSD, Wenzel JGW, *et al*. Analytical sensitivity of assays used for detection of bovine viral diarrhea virus in semen samples from the Southeastern United States. Vet Microbiol 2003;96(2):145-155.

36. Edmondson MA, Givens MD, Walz PH, Gard JA, Stringfellow DA, Carson RL. Comparison of tests for detection of bovine viral diarrhea virus in diagnostic samples. J Vet Diagn Invest 2007;19:376-381.

37. Grooms DL. Reproductive consequences of infection with bovine viral diarrhea virus. Vet Clin North Am Food Anim Pract 2004;20:5-19.

38. Hernández BEG, Gutiérrez HJL, Herrera LE, Palomares REG, Díaz AE. Frecuencia de diarrea viral bovina, rinotraqueitis infecciosa bovina, leptospirosis y brucelosis, en las 2 regiones ganaderas más importantes de Oaxaca. En: González IDR, Posadas ME editores. XXXIX Congreso Nacional de Buiatría. 2015:87-92.

39. Peña MA, Góngora A, Jiménez C. Infectious agents affecting fertility of bulls, and transmission risk through semen. Retrospective analysis of their sanitary status in Colombia. Rev Colomb Cienc Pecu 2011;24:634-646.

40. Oliveira MT, Campos FS, Dias MM, Velho FA, Freneau GE, Brito WMED, *et al*. Detection of bovine herpesvirus 1 and 5 in semen from Brazilian bulls. Theriogenology 2011;75:1139-1145.

41. Morán PE, Favier PA, Lomónaco M, Catena MC, Chiapparrone ML, Odeón AC, *et al*. Search for the genome of bovine herpesvirus types 1, 4 and 5 in bovine semen. Open Vet J 2013;3(2):126-130.

42. Gregersen JP, Wagner K. Persistent infection of the genital tract and excretion of the vaccine strain after live virus inmunization with bovine herpesvirus 1 (IBR/IPV virus). Zentralbl Veterinaermed B 1985;32:1-10.

43. Alonzo P, Puentes R, Benavides U, Esteves PA, Silva AD, Roehe PM, *et al*. Infección natural de un toro con dos subtipos diferentes de herpesvirus bovino tipo 1. Veterinaria (Montevideo) 2011;48(184):5-10.

44. Duque D, Estévez JNR, Abreu AM, Moncada M, Durango J, Molina D. Aspectos sobre rinotraqueitis infecciosa bovina. J Agric Anim Sci 2014;3(1):58-71.

45. Jain L, Kanani AN, Kumar V, Joshi CG, Purohit JH. Detection of bovine herpesvirus 1 infection in breeding bulls by ELISA and PCR assay. Indian J Vet Res 2009;18(1):1-4.

46. Sá Filho MFL, Vieira LCM, Martins CA, Rodríguez PS. New approaches in superovulation programs and embryo transfer in Brazil. Curso Internacional de Biotecnologías Reproductivas en Ganadería Tropical. 2013:1-9.